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# Two *Drosophila* nervous system antigens, Nervana 1 and 2, are homologous to the β subunit of $Na^+,K^+$-ATPase

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**ABSTRACT** **A nervous system-specific glycoprotein antigen from adult *Drosophila* heads, designated Nervana (Nrv), has been purified on the basis of reactivity of its carbohydrate epitope(s) with anti-horseradish peroxidase (HRP) antibodies that are specific markers for *Drosophila* neurons. Anti-Nrv monoclonal antibodies (mAbs), specific for the protein moiety of Nrv, were used to screen a *Drosophila* embryo cDNA expression library. Three cDNA clones (designated Nrv1, Nrv2.1, and Nrv2.2) were isolated that code for proteins recognized by anti-Nrv mAbs on Western blots. DNA sequencing and Southern blot analyses established that the cDNA clones are derived from two different genes. *In situ* hybridization to *Drosophila* polytene chromosomes showed that the cDNA clones map to the third chromosome near 92C-D. Nrv1 and Nrv2.1/2.2 have open reading frames of 309 and 322/323 amino acids, respectively, and they are 43.4% identical at the amino acid level. The proteins deduced from these clones exhibit significant homology in both primary sequence and predicted topology to the β subunit of $Na^+,K^+$-ATPase. Immunoaffinity-purified Nrv is associated with a protein ($M_r$ 100,000) recognized on Western blots by anti-ATPase α-subunit mAb. Our results suggest that the *Drosophila* nervous system-specific antigens Nrv1 and -2 are neuronal forms of the β subunit of $Na^+,K^+$-ATPase.**

All neurons in the central and peripheral nervous systems of *Drosophila melanogaster* and other insects express carbohydrate epitopes that are recognized by antibodies to the plant glycoprotein horseradish peroxidase (HRP) (1). Anti-HRP antibodies can stain the entire neuronal surface even at early stages of nervous system development in intact organisms, making these antibodies useful for tracing neural pathways (2–4). Neuronal precursor cells can also be stained in early stage *Drosophila* embryo cultures (5). Because anti-HRP antibodies recognize a nongenetically coded carbohydrate structure rather than protein (3, 4), the associated gene products have not been investigated extensively.

Recently, two anti-HRP reactive proteins ($M_r$ 42,000 and 80,000), which are likely to be the major contributors of neuronal anti-HRP staining in *Drosophila*, have been identified on Western blots (6). The $M_r$ 42,000 glycoprotein termed Nervana (nerve antigen; Nrv) was purified from adult *Drosophila* heads and used to prepare a panel of monoclonal antibodies (mAbs) that, depending on developmental stage, recognize two or three bands on Western blots between $M_r$ 38,000 and 42,000 (7). Immunocytochemical staining of *Drosophila* embryo cell cultures and whole-mount embryos using the mAbs indicates that the staining pattern is indistinguishable from anti-HRP staining.

These mAbs have now been used to screen a cDNA expression library made from *Drosophila* embryo mRNA. Two different cDNA clones have been isolated and both express proteins that are recognized by most of the anti-Nrv mAbs on Western blots. DNA sequence analyses revealed significant homology between the two clones designated Nrv1 and -2.† *In situ* hybridization to polytene chromosomes indicated that both clones map to the third chromosome near 92C-D. Southern blot hybridization of *Drosophila* genomic DNA using each clone as a probe indicated that they represent different genes. The proteins deduced from the nucleic acid sequence of both clones exhibit significant homology to the $Na^+,K^+$-ATPase β subunit of a variety of other species in both primary sequence and secondary structure. The β subunit of $Na^+,K^+$-ATPase is an invariant component of purified $Na^+,K^+$-ATPase (8) that may play a role in assembly and transport of active enzyme from cytoplasm to the plasma membrane (9, 10). Immunoaffinity-purified Nrv is associated with a protein of $M_r$ 100,000 that is recognized on Western blots by anti-*Drosophila* ATPase α-subunit mAb. Our results suggest that Nrv1 and Nrv2 are neuron-specific forms of $Na^+,K^+$-ATPase β subunit.

## MATERIALS AND METHODS

**cDNA Cloning and Sequencing.** Poly(A)$^+$ RNA from 12- to 24-hr-old Canton S *D. melanogaster* embryos was used to construct a cDNA library in the Lambda ZAP II vector (kindly provided by T. Kitamoto, Stratagene). This expression library was screened (11) with a mixture of anti-Nrv mAbs (7). Positive clones were plaque-purified and the phagemid (pBluescript, containing the inserts) was excised *in vivo* using the ExAssist/SOLR system (Stratagene). The inserts were sequenced (both strands) by the dideoxynucleotide chain-termination method of Sanger *et al.* (12) using the Sequenase 2.0 kit (United States Biochemical).

**Northern and Southern Blot Analysis.** For Northern blot analysis, *Drosophila* poly(A)$^+$ RNA (11) was electrophoresed in a 1.5% agarose gel and transferred onto a nitrocellulose membrane. Hybridization was carried out with a digoxigenin-labeled complementary RNA (cRNA) probe (Boehringer Mannheim) overnight at 50°C. The cRNA probe spanned nearly the entire coding region (see Fig. 1) and was visualized by incubation with sheep anti-digoxigenin IgG, conjugated to alkaline phosphatase, followed by color development with a bromochloroindolyl phosphate/nitroblue tetrazolium solution (13). For genomic Southern blot analysis, high molecular weight DNA, isolated from adult *Drosophila* (14), was digested with different restriction endonucleases, sized in a 0.8% agarose gel, and transferred onto nitrocellulose membrane. Hybridization and visualization were performed with a digoxigenin-labeled cRNA probe.

***In Situ* Hybridization to Polytene Chromosomes.** Salivary glands from late third-instar larvae were dissected in 45% acetic acid. Chromosome squashes and hybridizations were

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Abbreviations: HRP, horseradish peroxidase; mAb, monoclonal antibody; cRNA, complementary RNA.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U22438, U22439, and U22440).

performed according to De Frutos *et al.* (15) except that the RNase treatment was eliminated. Hybridization and visualization were with a digoxigenin-labeled cRNA probe.

**Immunoaffinity Purification.** Anti-Nrv mAb H7C4 or H5F7 (7) was bound to protein G-Sepharose 4 fast flow (Pharmacia) and then covalently cross-linked using the bifunctional coupling reagent dimethylpimelimidate (Sigma) (13). A deoxycholic acid extract of *Drosophila* heads (7) was applied to the affinity column followed by washing with 20 mM Tris·HCl buffer containing 0.01% Nonidet P-40 (pH 8.0). Proteins binding to the column were eluted with a low pH buffer and fractions were collected directly into tubes containing neutralization buffer. Fractions were analyzed for $Na^+,K^+$-ATPase $\alpha$-subunit protein and Nrv protein by Western blotting (see below).

**Western Blot Analysis.** The *Drosophila* head deoxycholic acid extract and immunoaffinity-purified fractions were mixed with SDS dissociation buffer, applied to 10% polyacrylamide slab gels, and electrophoresed (16). The separated proteins were electrophoretically transferred to nitrocellulose membrane and probed with anti-Nrv or anti-*Drosophila* $Na^+,K^+$-ATPase $\alpha$ subunit ($\alpha_5$-IgG; a kind gift from D. M. Fambrough, Johns Hopkins) mAbs (7, 17). Antibody binding was detected with alkaline phosphatase-labeled goat anti-mouse IgG and the color was developed with a bromochloroindolyl phosphate/nitroblue tetrazolium solution (13).

## RESULTS

**Isolation and Characterization of Nrv cDNA Clones.** The *Drosophila* embryonic cDNA expression library was screened with a mixture of anti-Nrv mAbs (H7C4, H5F7, and H2H11) (7). Two positive clones were isolated with inserts of 1.5 and 1.6 kb. Both clones expressed proteins that were recognized on Western blots by 17 of 20 anti-Nrv mAbs (data not shown). The inserts were subcloned into a plasmid vector (pBluescript II SK) for restriction mapping and sequencing. From the size of the deduced proteins neither insert was full length (they lacked the 5′ end). Therefore, we rescreened the embryonic cDNA library using the cRNA probes transcribed from the two positive clones. We have now isolated three full-length cDNA clones with inserts of 2.2, 2.0, and 2.1 kb, which are schematically shown in Fig. 1. We have designated these clones Nrv1, Nrv2.1, and Nrv2.2, respectively. Nrv2.1 and Nrv2.2 are transcripts of the same gene with different 5′ ends resulting from differential RNA splicing.

Nrv1 has an open reading frame of 309 amino acids, assuming that the first AUG is the translation initiation codon. Nrv2.1 and Nrv2.2 have 322 and 323 amino acids, respectively.

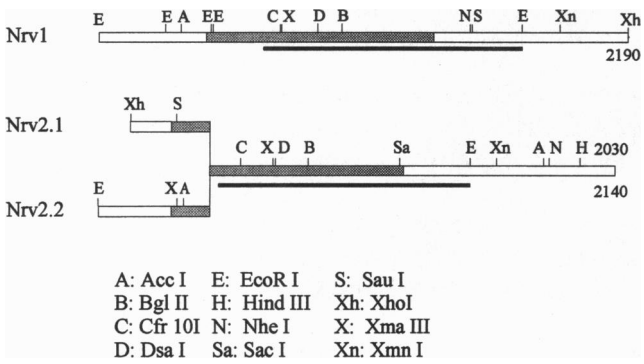


FIG. 1. Schematic of Nrv1, Nrv2.1, and Nrv2.2 cDNA clones. Shaded box, putative translated region; open box, untranslated region. Solid line under each clone, transcribed region used to prepare cRNA probes. Number of nucleotides in each clone is indicated on the right. Positions of restriction sites are indicated by one or two letters along each clone and are defined at the bottom.

Nrv1 and Nrv2 (represented by Nrv2.1) are 58.6% identical at the DNA level and 43.4% identical and 54.7% similar at the amino acid level. One feature of the comparative amino acid sequences of Nrv1 and -2 is the presence of four peptide fragments containing 14, 10, 9, and 7 contiguous amino acid identities (see below). In addition, both proteins contain many smaller regions of identity and similarity and have 7 cysteines in conserved positions. Nrv1 and Nrv2 also have two potential N-glycosylation sites; however, the positions are not conserved.

A Nrv1 cRNA probe hybridized to a major band located at 2.2 kb and to a weaker band at 2.0 kb using *Drosophila* poly(A)$^+$ RNA in Northern blot analysis (Fig. 2*A*), while a Nrv2 probe hybridized to a single band at 2.0 kb. The 2.2-kb Nrv1 band and the 2.0-kb Nrv2 band are the same size as the cDNA inserts.

**Nrv1 and -2 Are Separate Genes.** Southern blot analysis of *Drosophila* genomic DNA digested with three different restriction enzymes (*Bam*HI, *Eco*RI, and *Xho* I) shows the presence of unique fragments for Nrv1 and Nrv2 (Fig. 2*B*). The blots were probed with cRNA probes as shown in Fig. 1 and the results indicate that Nrv1 and Nrv2 are derived from different genes.

A digoxigenin-labeled cRNA probe for each cDNA was used in *in situ* hybridization to *Drosophila* polytene chromosomes to determine the cytogenetic locus of the Nrv1 and Nrv2 genes. Both probes hybridized to single positions on the right arm of the third chromosome near 92C-D (Fig. 3). When both probes were hybridized together, the signal appeared as two closely spaced bands (Fig. 3 *Inset*).

**Nrv1 and -2 Are Related to the $\beta$ Subunit of $Na^+,K^+$-ATPase.** A comparison of the deduced amino acid sequences of Nrv1 and Nrv2 with all the sequences in the Swiss-Prot 28 and Protein Identification Resource 40 protein sequence data bases showed homology to the $\beta$ subunit of $Na^+,K^+$-ATPase from a variety of vertebrate and invertebrate species. A comparison of the deduced amino acid sequences of Nrv1 and Nrv2 to each other as well as to the $Na^+,K^+$-ATPase $\beta$ subunit of brine shrimp (*Artemia*; ref. 18) is shown in Fig. 4. The brine shrimp $\beta$ subunit consists of 315 amino acid residues and

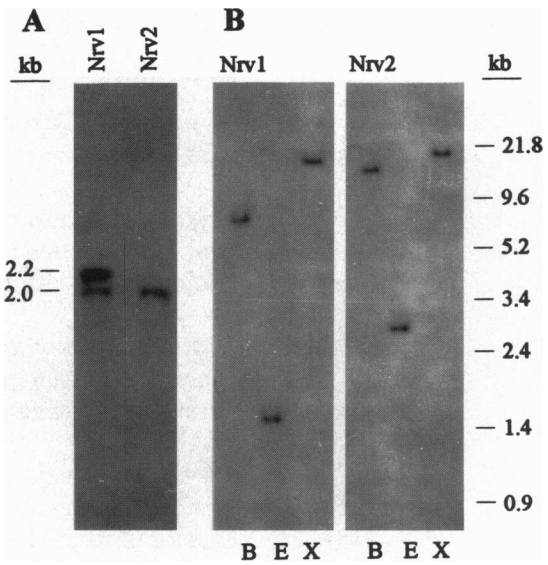


FIG. 2. Northern and Southern blot analysis of Nrv1 and Nrv2.1/2.2. (*A*) *Drosophila* poly(A)$^+$ RNA (15 $\mu$g) from third-instar larvae was hybridized with either a Nrv1 or a Nrv2.1/2.2 cRNA probe. Sizes of mRNA bands calculated from RNA standards are shown on the left. (*B*) *Drosophila* genomic DNA (15 $\mu$g) was digested with different restriction enzymes (B, *Bam*HI; E, *Eco*RI; X, *Xho* I). Hybridization was performed with Nrv1 or Nrv2.1/2.2 cRNA probes. Sizes of DNA markers are indicated on the right.

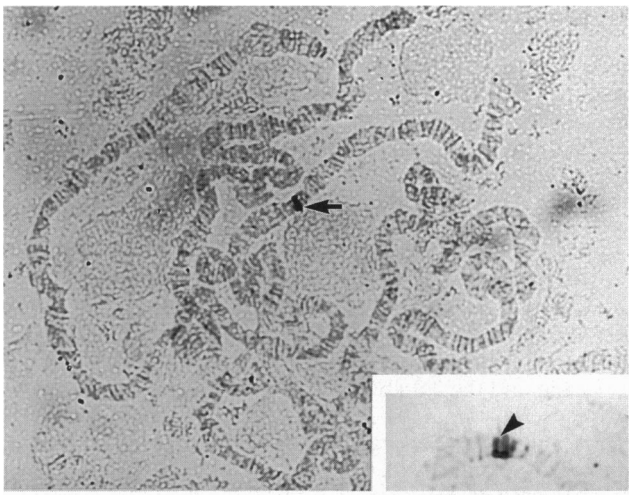

FIG. 3. *In situ* hybridization of Nrv1 and Nrv2.1/2.2 cDNA to *Drosophila* salivary gland chromosomes. Salivary chromosomes from third-instar larvae were hybridized with digoxigenin-labeled Nrv1 and/or Nrv2.1/2.2 cRNA probes. Both probes hybridized to a single position on the right arm of the third chromosome near 92C-D (arrow). In some preparations using both probes together (*Inset*), the hybridization appears as two closely spaced bands (arrowhead).

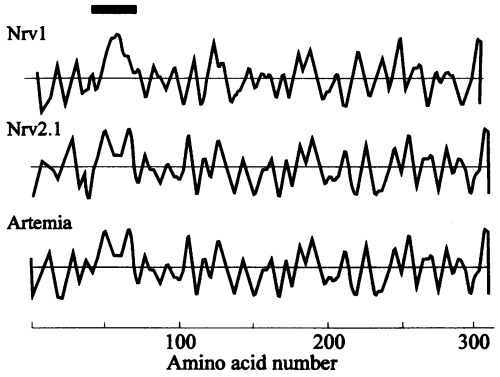


FIG. 5. Comparison of the hydropathy profiles of Nrv1, Nrv2.1/2.2, and the $Na^+,K^+$-ATPase $\beta$ subunit of brine shrimp (*Artemia*). Hydropathy plots were obtained by using the algorithm of Kyte and Doolittle (20). Hydrophobic regions are above the center line and hydrophilic regions are below. Solid bar represents predicted transmembrane region.

29.4% of the positions were occupied by identical residues, while 11.0% were occupied by conservative substitutions. In addition, 6 conserved cysteine positions were found in all three sequences, which correspond to those known to be involved in disulfide bond formation (19). A pairwise comparison of the three sequences revealed that Nrv2 is 60% homologous (identical and conservative amino acids) with brine shrimp $\beta$ subunit, while Nrv1 is only 53% homologous with the brine shrimp sequence. Nrv2 thus shows greater homology to the brine shrimp $\beta$-subunit sequence than to Nrv1 (55%). Among the three potential N-linked glycosylation sites identified in the brine shrimp $\beta$ subunit, only one (position 198) is found in a conserved position in Nrv2 (position 205; Fig. 4).

Hydropathy profiles of Nrv1, Nrv2, and brine shrimp $\beta$-subunit sequences obtained by using the algorithm of Kyte and Doolittle (20) are shown in Fig. 5. This analysis predicts that both Nrv1 and Nrv2 have a single hydrophobic transmembrane domain of 20 amino acids near the N terminus. The predicted secondary structures of Nrv1 and Nrv2 appeared very similar to each other as well as to that of the brine shrimp $\beta$ subunit.

The protein sequences of Nrv1 and Nrv2 also show homology to the $Na^+,K^+$-ATPase $\beta$ subunits of higher organisms—e.g., sheep (21), dog (22), rat (23), human (24), and several other vertebrates (25–27). Amino acid comparisons among mammalian species have revealed >90% similarity for $\beta$-subunit sequences. Both Nrv1 and Nrv2 protein sequences show ≈46% similarity and ≈30% identity with mammalian $\beta$ sub-

```
                    M
NRV1     MSKNNGKGAKGEF---EFPQPAKKQTF--SEMIYNPQEGTFFGRTGKSWSQLLLF 50
NRV2.1   MSKPVPMSP--SFVDEDLHNLRKPKPFKLGQFLYNTEDGTVMGRDRSSWAKIGIF 53
ARTEMIA  MADKKP-DE--QFVGSG-PKETKWQSFK--GFVWNSETSQFMGRTAGSWAKITIF 49

         YTIFYIVLAALFTICMQGLLSTISDTEPKWKLQDSLIGTNPGLGFRPLSEQTERG 105
NRV1     YVAFYGVLAALVAICMWAFFQTLDPRIPKWTLDRSLIGTNPGLGFRPLPPVDNVE 108
NRV2.1   YVIFYTLLAGFFAGMLMIFYQTLDFKIPKWQNKDSLIGANPGLGFRPMPPEAQVD 104
ARTEMIA

         S-VIAFDGKKPAESDYWIELIDDFLRDYN-----HTEGRDMKHCGFGQVLEPTDV 154
NRV1     STLIWYKGTQHENYKHWTDSLDDFLAVYKVPGLTPGRGQNIYNCDYNQPPPKAQV 163
NRV2.1   STLIQFKHGIKGDWQYWVHSLTEFLEPYET--LTSS-GQEFTNCDFDKPPQEGKA 156
ARTEMIA

         CVVNTDLFGG-CSKANNYGYKTNQPCIFLKLNKIFGWIPEVYDKEEK---DMPDD 205
NRV1     CDVDIKTWS-PCTKENNYSYHKSAPCIFLKLNKIYGWIPEYYNRSNDLPANMPAS 217
NRV2.1   CNFNVELLGDHCTKENNFGYELGKPCVLIKLTD-FGWRPEVYNSSAEVPEDMPAD 210
ARTEMIA

         LKKVINETKTEERHE---VWVSCFGHLGKDKENFQNIRYFPSQGFPSYYYPFLNQ 257
NRV1     LKTYIAEVEKTQPEKLNTIWVSCEGENPADQENIGAVNYLPIRGFPGYFYPYQNS 272
NRV2.1   LKSYIKDIETGNKTHMNMVWLSCEGETANDKEKIGTITYTPFRGFPAYYYPYLNV 265
ARTEMIA

         PGYLSPLVAVQFNSPPKGQMLDVECRAWAKNIQYSGSARDRK---GSVTFQILLD 309
NRV1     EGYLSPLVAVHFQRPKRGIIINVECRAWARNI-----IRDRKERIGSVHYELLID 322
NRV2.1   PGYLTPVVALQFGSLQNGQAVNVECKAWANNI-----SRDRQRRLGSVHFEIRMD 315
ARTEMIA
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FIG. 4. Comparison of the predicted amino acid sequences of Nrv1, Nrv2.1/2.2, and the $Na^+,K^+$-ATPase $\beta$ subunit of brine shrimp (*Artemia*; ref. 18). Amino acids identical in two or three sequences are shaded. Putative transmembrane regions are indicated by thick black overline and conserved cysteine positions are indicated by arrows. Potential N-linked glycosylation sites are indicated by underlining.

units. Rats contain two different forms of $\beta$ subunit with less homology between them (28). For example, the rat $\beta_2$ subunit exhibits only 53% homology with the $\beta_1$ subunit of rat (28). This compares well with the 55% homology between Nrv1 and Nrv2. A pairwise comparison of the protein sequences of Nrv1 and Nrv2 with rat $\beta_1$ and $\beta_2$ subunits reveals homology between them as follows: Nrv1/rat $\beta_1$, 47%; Nrv1/rat $\beta_2$, 44%; Nrv2/rat $\beta_1$, 41%; Nrv2/rat $\beta_2$, 42%. Transcripts for the rat $Na^+,K^+$-ATPase $\beta_1$ subunit can be detected in the nervous system as well as other nonneuronal tissue(s) (28). The $\beta_2$-subunit mRNA, however, is preferentially expressed in glial cells of the nervous system (29). $Na^+,K^+$-ATPase and $H^+,K^+$-ATPase are homologous members of the P-type ATPase family, which have glycosylated $\beta$ subunits (30). Nrv1 and -2 appear more closely related to the $Na^+,K^+$-ATPase $\beta$ subunits ($\approx$30% identity) compared to $H^+,K^+$-ATPase $\beta$ subunits (22–26% identity).

**Nrv Is Copurified with *Drosophila* $Na^+,K^+$-ATPase $\alpha$ Subunit.** Nrv1 and/or Nrv2 can be copurified with the $\alpha$ subunit of *Drosophila* $Na^+,K^+$-ATPase. When a deoxycholic acid extract of *Drosophila* heads is chromatographed on an anti-Nrv mAb immunoaffinity column, three major protein bands can be eluted at low pH. The two faster migrating bands ($M_r \approx$ 42,000) are recognized by anti-Nrv mAb staining on Western blots (Fig. 6). The slower migrating band ($M_r \approx$ 100,000) is not stained by anti-Nrv mAb, but it is stained by an anti-*Drosophila* $Na^+,K^+$-ATPase $\alpha$-subunit mAb ($\alpha_5$-IgG; ref. 18; Fig. 6). The anti-$\alpha_5$ staining band is also observed in a lentil lectin affinity purification of Nrv (ref. 7; data not shown). The *Drosophila* $Na^+,K^+$-ATPase $\alpha$ subunit is thus closely associated with Nrv during immunoaffinity chromatography using an anti-Nrv mAb or lentil lectin chromatography.

## DISCUSSION

In this report, we have documented the existence of two separate genes encoding protein products recognized by several different anti-Nrv mAbs. The two gene products, termed Nrv1 and Nrv2.1/2.2, are homologous to each other at the level of amino acid sequence and secondary structure. They contain several regions of amino acid identity and similarity consistent with their common reactivity to 17 of 20 different mAbs. None of our anti-Nrv mAbs can distinguish Nrv1 from Nrv2 protein; however, it is possible to distinguish unique transcripts on Northern blots by using Nrv1- or Nrv2-specific probes.

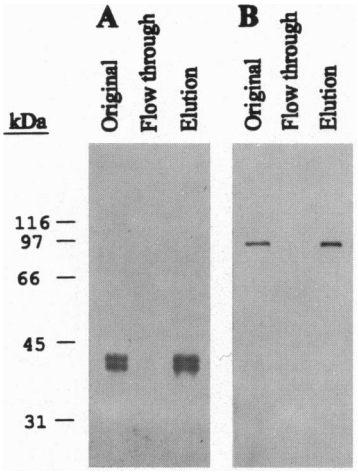


FIG. 6. Western blot analysis of immunoaffinity-purified Nrv and $Na^+,K^+$-ATPase $\alpha$ subunit. A deoxycholate extract of *Drosophila* heads (Original), anti-Nrv immunoaffinity column flow through (Flow through), and immunoaffinity-purified fraction (Elution) were size-fractionated on SDS/10% polyacrylamide gels, transferred onto nitrocellulose filters, and probed with anti-Nrv mAb H7C4 (*A*) or a mAb against *Drosophila* $Na^+,K^+$-ATPase $\alpha$ subunit ($\alpha_5$-IgG) (*B*). Molecular size markers are shown on the left.

Also apparent from the nucleic acid and predicted amino acid sequences of Nrv1 and Nrv2 is their homology to the $Na^+,K^+$-ATPase $\beta$ subunits from a variety of other species. The homology includes regions of identical and conserved amino acid residues, predicted secondary structure, the position of a single putative transmembrane domain, and the positions of 6 cysteine residues. *Drosophila* $Na^+,K^+$-ATPase $\alpha$ subunit copurifies with Nrv, indicating a close association between the two proteins. Nrv1 and Nrv2 are thus different forms of *Drosophila* $Na^+,K^+$-ATPase $\beta$ subunit.

$Na^+,K^+$-ATPase is the enzyme responsible for maintenance of the $Na^+$ and $K^+$ gradients present in most eukaryotic cells. It couples the energy of ATP hydrolysis with expulsion of intracellular $Na^+$ and entry of extracellular $K^+$ against concentration gradients. It is thought to be present in the plasma membranes of all types of eukaryotic cells and plays a critical role in the nervous system, where it contributes to the electrogenic properties of neurons (31, 32).

Nrv was originally isolated as an $M_r$ 42,000 protein, which could be identified as the major glycoprotein component in *Drosophila* recognized by anti-HRP antisera (7). Anti-HRP staining is restricted to neurons and a few other tissues in *Drosophila* and other insects (1–4) and is used extensively as a specific neuronal marker. mAbs that recognize protein epitopes of Nrv stain two or three components on Western blots of *Drosophila* tissue extracts and stain *Drosophila* embryos and embryonic cell cultures with a pattern indistinguishable from anti-HRP staining (7). The cellular distribution pattern of Nrv protein is primarily specific for neurons, and Nrv is therefore a neuron-specific form of $Na^+,K^+$-ATPase $\beta$ subunit. Other species also contain multiple forms of $Na^+,K^+$-ATPase $\beta$ subunit (28, 29, 33, 34), although neuron-specific forms have not yet been described. AMOG, the rat $Na^+,K^+$-ATPase $\beta_2$ subunit, is preferentially expressed by glial cells in the nervous system (33, 35), while several other studies have established the tissue-specific and in some cases cell type-specific expression of the catalytic $Na^+,K^+$-ATPase $\alpha$ subunit in a variety of species (29, 36, 37). It is somewhat surprising that we do not detect Nrv1 or Nrv2 expression in other types of *Drosophila* tissue, such as muscle, which also contains $Na^+,K^+$-ATPase (38). Perhaps another type of $\beta$ subunit exists in *Drosophila* or the levels of Nrv1 and/or Nrv2 expression may be below the sensitivity of our immunocytochemical assay.

The function of the $\beta$-subunit of $Na^+,K^+$-ATPase is largely unknown. It is known to participate in delivery of active $\alpha$ subunit to the plasma membrane (9, 10). The $\beta_2$ subunit in glial cells (AMOG) can also participate in mediating cell–cell interactions (39, 40). Null mutations in AMOG/$\beta_2$ subunit result in somewhat surprising neuronal degeneration phenotype in the central nervous system (41). Recently, the rodent $Na^+,K^+$-ATPase $\alpha$ subunit, expressed independently of $\beta$ subunit in a baculovirus system, showed properties of a $Mg^{2+}$-ATPase, suggesting a role for $\beta$ subunit in determining the cation specificity of the enzyme (42). We do not know the precise function(s) of Nrv1 and Nrv2 or whether they are present in plasma membrane independently of $Na^+,K^+$-ATPase $\alpha$ subunits. *Drosophila*, however, offers the possibility of generating mutants and using genetics to further characterize $\beta$-subunit function. Nrv mutations can also be combined with $\alpha$-subunit mutants. A hypomorphic *Drosophila* $Na^+,K^+$-ATPase $\alpha$-subunit mutant has recently been described (43) that shows the interesting nervous system phenotype of bang sensitivity.

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